

# The Effect of Acute Beetroot Juice Supplementation After Strenuous Physical Activity on Creatine Kinase Levels

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## The Effect of Acute Beetroot Juice Supplementation After Strenuous Physical Activity on Creatine Kinase Levels

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**Abstract.** Strenuous physical activity can cause muscle damage which is marked by an increase in creatine kinase levels. One of the strategies to prevent muscle damage is to consume antioxidants and anti-inflammatory. Beetroot (*Beta vulgaris* L.) is known to have antioxidant and anti-inflammatory activity. The aim of this study was to determine the effect of acutely giving beetroot juice on creatine kinase levels after strenuous physical activity. The research sample used 20 trained males. The sample was divided into two groups (Experiment = 10; Placebo = 10). The pre-test was done by checking creatine kinase levels before engaging in strenuous physical activity. After doing strenuous physical activity by running on a treadmill with an intensity of 90-100% for 30 minutes, the experimental group was given 300 ml of beetroot juice (BRJ) every day for 3 days, while the control group was given a placebo drink. Creatine kinase levels were measured immediately, 24 hours, 48 hours, and 72 hours after strenuous physical activity. The results showed that CK levels decreased significantly 24 hours, 48 hours and 72 hours after strenuous physical activity in the BRJ group compared to the placebo group ( $p < 0.05$ ). The conclusion of this study is that giving BRJ acutely after doing strenuous physical activity can reduce creatine kinase levels in trained male athletes. Acute IBR supplementation in this study can help reduce muscle damage due to strenuous physical exercise through nutritional interventions

### 1. Introduction

Regular physical exercise, especially strenuous physical exercise, can induce muscle damage. Exercise-Induced Muscle Damage (EIMD) is characterized by symptoms that appear immediately and for up to 14 days after exercise [1]. Exercise-Induced Muscle Damage for athletes will have a direct impact on functional capacity, muscle pain [2], training capacity [3] and impaired sense of strength production and impaired sense of strength and leg position [4].

One indicator of muscle damage triggered by strenuous physical exercise is an increase in creatine kinase levels [5,6]. Creatine kinase consists of two sub units, namely B (brain) and M (muscle), each sub unit has a molecule weighing 43,000 Daltons. So the combination of these two sub-units will only produce three creatine kinase isoenzymes, namely CK-BB (CK-1), CK-MB (CK-2), and CK-MM (CK-3). CK-BB can mainly be found in the kidneys and brain while CK-MM is mostly found in skeletal muscles [6]. Creatine kinase (CK) is a protein involved in muscle metabolism, and its concentration is generally used as a marker of physical stress triggered by exercise [7]. Creatine kinase (CK) and lactate dehydrogenase (LDH) are fragments of the myosin heavy chain and are related to muscle damage [8].

Various methods are used to minimize exercise induced muscle damage that is triggered by strenuous physical activity, including by administering anti-inflammatory drugs [9-12], massage [13-



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15]electrical therapy[16–18], nutritional[19–25] and cryotherapy[26,27]. Several studies that use nutrition to minimize the side effects caused by exercise-induced muscle damage include giving curcumin[22], grapes[23], cherry[6][20], watermelon[21] and pomegranate [24]. In general, the mechanism of action of nutrition and supplementation to prevent and reduce exercise-induced muscle damage is due to antioxidant and anti-inflammatory activities [28]. One of the natural ingredients that has antioxidant and anti-inflammatory effects is beets (*Beta vulgaris* L.) Beetroot contains important phytochemical compounds such as flavonoids, ascorbic acid, phenolic acids, carotenoids [29] as well as a group of bioactive pigments known as betalain[30]. Betalain, which is a compound that has very high antioxidants that can neutralize free radicals [31]. Additionally, beetroot is known to contain high amounts of nitrates[32], which can be converted into nitric oxide (NO) after consumption [33]. The results of the study reported that nitrite and nitric oxide were shown to suppress radical formation and extinguish potentially damaging reactive oxygen (ROS) and nitrogen (RNS) species, suggesting that nitrates may also exhibit antioxidant effects [34,35]. The specialty of beetroot which contains nitrates and has antioxidant and anti-inflammatory properties made researchers interested in evaluating the effect of acute beet juice supplementation after strenuous physical activity on CK levels measured up to 72 hours after exercise.

## 2. Methods

### 2.1. Participants

Twenty trained males (age  $21.25 \pm 1.48$  years, height  $170.50 \pm 0.04$  cm, weight  $64.00 \pm 4.17$  kg) volunteered to participate in this study. The criteria for the participants were not suffering from any acute or chronic illnesses and not being injured for at least three months before the start of the study. Prior to the study, socialization was carried out by instructing participants to avoid using drugs (anti-inflammatory drugs and antioxidants) two months before the study. Apart from drugs, it is also avoided consuming foods or drinks that contain antioxidants such as green tea, curcuma, red wine, blueberries, coffee, and cherries at least two months before the study begins.

### 2.2. Ethical Clearance

Participants are informed about the research procedure, the potential risks arising from the activities carried out, until all participants give written consent as a means of agreement to follow the research procedure. Research was also carried out in accordance with the declaration of Helsinki (this research was approved by the ethics committee of the University of North Sumatra No: 277 / KEP / USU / 2020) before the study began

### 2.3. Exercise Program

The study used 20 futsal athletes who met the criteria. Before undergoing the exercise program, a haematology examination was carried out to measure the concentration of CK (Pre-test). Furthermore, the sample was divided into 2 groups, namely the experimental group who drank 300 ml of beetroot juice (BRJ) once per day in the evening up to 72 h after strenuous physical activity and the control group who drank a placebo. Strenuous physical activity is done by running on a treadmill with an intensity of 90-100% of the maximum heart rate. After performing strenuous physical activity, blood samples were taken to re-check the CK concentration every 24 hours for 3 days (Post-test).

### 2.4. Blood sampling

Blood was drawn before the participants did maximum physical activity by inserting a needle into the antecubital vein of each participant's forearm and blood was drawn as much as 5ml. Furthermore, the blood sample was centrifuged for 10 minutes with 3000 rotations to separate the serum. The serum is then stored at  $-80^{\circ}\text{C}$ . Then the serum CK level was measured using an auto-analyzer spectrophotometer. Blood draws to measure CK levels were again performed after 24 hours, 48 hours and 72 hours of the participants doing maximum physical activity.



### 2.5. Statistical analysis

All data are expressed as mean  $\pm$  standard deviation (SD) and were analysed using IBM SPSS Statistics 25 for Windows. Data normality test was done by using the Shapiro Wilk test method and data homogeneity by using the Levene's test. Repeated-measure analysis of variance was conducted to examine the interaction effect by time and group in this study. Posthoc analysis was performed using the Tukey method. The level of significance was set at  $P < 0.05$ .

### 3. Results

The results of measurements of CK shown in Table 1. Based on the research results obtained pretest CK levels in the treatment group were  $137.4 \pm 9.0$  U/L, while the control group  $138.8 \pm 9.3$ U/L. The results of statistical tests showed that there was no difference in CK levels between the experimental group (BRJ) and the control group (Placebo). After performing strenuous physical activity, there was an increase in CK levels after 24 hours, 48 hours in both the BRJ and placebo groups. The decrease in CK levels was obtained after 72 hours in both the BRJ group and the placebo group. The results of statistical tests with the independent t test showed that there were differences in CK levels between the BRJ group and the placebo group at each time of the CK measurement, where the CK levels in the Placebo group were greater than the BRJ group ( $p = 0.000$ )

**Table 1.** Changes in creatine kinase level after strenuous physical activity.

Group	Pre (U/L)	24h (U/L)	48h (U/L)	72h (U/L)	P
BRJ (n=10)	137.4 $\pm$ 9.0	152.6 $\pm$ 9.6	163.8 $\pm$ 10.2	137.9 $\pm$ 3.3	0.000
Placebo (n=10)	138.8 $\pm$ 9.3	217.4 $\pm$ 10.4*	495.5 $\pm$ 6.2*	488.8 $\pm$ 5.6*	0.000

Values are mean  $\pm$  standard deviation.

Analysed using repeated-measure analysis of variance.

\*Significant between groups at 24h, 48h, 72 h, analysed by the independent sample t-test ( $p < 0.05$ ).

### 4. Discussion

This study investigated the effect of acute JBR supplementation (300 ml/day) on the muscle damage index by measuring CK levels. The results of this study indicated that CK levels decreased significantly with acute JBR supplementation after strenuous physical activity compared to the control group (placebo). It is known, physical activity, especially eccentric muscle contraction with high intensity, will induce muscle damage (Exercise-induced Muscle damage) [1]. Exercise-induced muscle damage (EIMD) can cause delayed onset muscle soreness (DOMS), decreased ability to generate muscle strength, range of motion (ROM), localized swelling [5] and an increase in muscle protein in the blood creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (Mb) [36].

The increase in CK levels in this study was supported by several other studies including the research of Callegari et al [37] which reported the effects of exercise resistance and aerobic exercise with different intensities to increase CK levels. In his study reported aerobic exercise was performed in 80% VO<sub>2</sub>max appears to increase plasma CK levels more than bi-set resistance exercise sessions. Increased levels of CK were also reported by the study of Natalie et al [38] evaluating muscle damage, body temperature, peak torque, and fatigue index in three different methods of strength gain. In their study, biochemical indicators of muscle damage, thermographic images, and neuromuscular performance were compared after athletes performed the traditional resistance training (TRAD), time under tension (TUT) and occlusion (OCL) methods with the lower limbs. The results showed that the peak muscle torsion decreased, CK and LDH levels increased and post-intervention skin temperature decreased in the TUT and OCL training methods.

Our findings suggest that acute administration of beet juice after strenuous physical activity can reduce the increase in CK levels. The decrease in CK levels in this study was probably due to the antioxidant and anti-inflammatory activity of beetroot juice. It is known that beetroot is a rich source of phytochemical compounds including ascorbic acid, phenolics (flavonoids, phenolic acids, phenolic amides), carotenoids and also contains bioactive pigments known as betalains. Betalains consists of

two groups namely betacyanins and betaxanthins[29,31,39]. Betalains contain up to ~70-100 percent of beetroot phenolic composition, limited to 0.8-1.3 g/L of fresh beetroot juice [40]. Several studies have reported betalains to have pharmacological effects as antioxidants and anti-inflammatory[30,41,42]. Betalains have potent free radical and anti-oxidant scavenging operations. Their scavenging behavior is similar to butylated hydroxytoluene, the synthetic anti-oxidant commonly used[43]. Research results reported, betalains mechanistically mitigated tumour necrosis factor-alpha (TNF-alpha), interleukin (IL)-1 $\beta$ , superoxide anion levels, and lipid peroxidation caused by carrageenin. Betalains, as well as upregulated Nrf2 and Ho1 transcript expression in the plantar tissue of mice, also stopped the depletion of decreased glutathione (GSH) levels and ferric reduction capacity induced by carrageenin[44]. Other compounds that act as powerful antioxidants in beetroot include betaine[45,46]. Betaine works as an anti-inflammatory by suppressing nuclear factor kappa-light chain enhancer of activated B cells (NF- $\kappa$ B) and Akt activation[45].

Additionally, beetroot is known to contain high amounts of nitrates[47,48]. The concentration of nitrates in beetroot juice was said to be within the range of  $388 \pm 19.9$  to  $3968 \pm 252$  mg/L. Nitrate can be converted into NO<sub>2</sub> through a bacterial enzymatic pathway (NO<sub>3</sub> reductase), which is then non-enzymatically broken down into NO in the oral cavity [49]. The results of the study reported that nitrite and nitric oxide were shown to suppress radical formation and extinguish potentially damaging reactive oxygen (ROS) and nitrogen (RNS) species, suggesting that nitrates may also exhibit antioxidant effects [34,35].

Our results are supported by the results of a study by Clifford et al [50] who reported the effect of beet juice supplementation on the index of muscle damage after eccentric exercise. In his study reported an increase in CK levels in the placebo group after eccentric exercise ( $350 \pm 28$ ;  $679 \pm 512$ ;  $533 \pm 384$ ;  $543 \pm 443$  U/L) during postexercise measurement, 24 h post exercise, 48 h post exercise and 72 h post exercise while the group drinking 250 ml of juice the increase in CK levels was ( $218 \pm 110$ ;  $3$ ;  $572 \pm 442$ ;  $431 \pm 260$ ;  $319 \pm 217$  U/L). Likewise, Montenegro et al. [51] reported that juice beet supplementation (100 mg / day for 7 days) reduced the increase in creatine kinase levels in the 10-km running time trial in male and female competitive triathletes.

## 5. Conclusion

In conclusion, giving juice beetroot acutely after doing strenuous physical activity can reduce creatine kinase levels in trained male athletes. Acute juice beetroot supplementation in this study can help reduce muscle damage due to strenuous physical exercise through nutritional interventions.

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